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Application No. 940292

Date of filing 6 April, 1994

Applicants ELAN CORPORATION, PLC, an Irish company of, Monksland Industrial Estate, Athlone, Co. Westmeath, Ireland.

PRIORITY DOCUMENT

Dated this 20TH day of April, 1995

V. Cunningham

An officer authorised by the
Controller of Patents, Designs and Trade Marks.

FORM NO. 1

940292

REQUEST FOR THE GRANT OF A PATENT

PATENTS ACT, 1992

The Applicant(s) named herein hereby request(s)

[x] the grant of a patent under Part II of the Act

[ ] the grant of a short-term patent under Part III of the Act

on the basis of the information furnished hereunder.

1. Applicant(s)

ame ELAN CORPORATION, PLC

Address Monksland Industrial Estate,
Athlone,
County Westmeath,
Ireland.

Description/Nationality an Irish company.

2. Title of Invention

BIODEGRADABLE MICROCAPSULES AND METHOD FOR THEIR MANUFACTURE

3. Declaration of Priority on basis of previously filed application(s) for same invention (Sections 25 & 26)

evious filing date	Country in or for which filed	Filing No.

4. Identification of Inventor(s)

Name(s) of person(s) believed by Applicant(s) to be the inventor(s)

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CONTINUED OVER

5. Statement of right to be granted a patent (Section 17(2)(b))

The Applicant has acquired the right to be granted a patent from the inventors by virtue of a Deed of Assignment dated April 5, 1994.

6. Items accompanying this Request - tick as appropriate

(i) [x] Prescribed filing fee (£117.00)

(ii) [x] Specification containing a description and claims
[ ] Specification containing a description only
[x] Drawings referred to in description or claims

(iii) [x] An abstract

(iv) [ ] Copy of previous application(s) whose priority is claimed

(v) [ ] Translation of previous application whose priority is claimed

(vi) [ ] Authorisation of Agent (this may be given at 8 below if this Request is signed by the Applicant(s))

7. Divisional Application(s)

The following information is applicable to the present application which is made under Section 24 -

Earlier Application No: .....
Filing Date: .....

8. Agent

The following is authorised to act as agent in all proceedings connected with the obtaining of a patent to which this request relates and in relation to any patent granted -

Name	Address
ANNE RYAN & CO.	60 Northumberland Road, Ballsbridge, Dublin 4, Ireland.

9. Address for Service (if different from that at 8)

ELAN CORPORATION, PLC

Signed By: Anne Ryan
ANNE RYAN - Their Attorney

Date April 6, 1994.

Biodegradable microcapsules and method for their manufacture

This invention relates to the preparation of biodegradable microsphere systems containing low molecular weight hydrophilic drugs. In particular, this invention relates to a formulation and a method for making this formulation, particularly an effective once-daily formulation, containing a microencapsulated hydrophilic drug such as a calcium antagonist, for example, nifedipine, a narcotic analgesic, for example, hydromorphone and an ACE-inhibitor, for example, captopril, and the like and analogues or mixtures thereof.

Normal drug dosing may follow a profile of excessive-ineffective plasma levels whereby the dose initially exceeds the desired therapeutic level, and then falls to subclinical levels. Controlled/sustained drug delivery reduces undesirable fluctuations of drug levels, promotes therapeutic benefits and minimises toxic effects. Pre-programmed drug delivery of a drug in the vicinity of its target cells can prevent systemic or side effects involving other tissues. To avoid inconvenient insertion of large implants, injectable or orally/rectally delivered biodegradable microparticles/microspheres appear to be the ideal delivery system.

Spherical microspheres of less than 250 µm, preferably less than 125 µm, in diameter are suitable for injection. It has been shown that orally administered particles are taken up from the intestine into Peyers' patches. Although the parameters controlling uptake are poorly understood, studies have shown the importance of particle size on particle uptake (O'Hagan, Adv. Drug Del. Rev., *5*:265-285 (1990)). Jani *et al.*, J. Pharmaceu. Pharmacol., *41*:809-812 (1989) claimed that optimal particle uptake was observed with particles 1 µm in diameter or below.

Microparticulate drug delivery systems based on biodegradable polymers, such as those formed from lactic and glycolic acid, have been widely studied for the controlled/sustained delivery of low

molecular weight drugs (U.S. Patent No. 4,952,402; Beck *et al.* Adv. in Human Fertility and Reproductive Endocrin., *2*:175-199 (1983); Benital *et al.*, Pharm. Sci., *73*:1721-1724 (1984); Benoit *et al.*, J. Pharm. Belg., *41*:319-329 (1986); and Bodmeier *et al.*, J. Control. Release, *15*:65-77 (1991)). The method most frequently used for the preparation of biodegradable microsphere systems is solvent evaporation from an oil-in-water emulsion. This method is not ideal for the entrapment of water soluble drugs as the drug is lost to the external phase. Furthermore, selection of the particular polymer(s) in light of the particular drug properties, as well as the manufacturing processes and process variables, determine the morphology and size of the microspheres, which in turn influence release characteristics for the formulation. These factors, which sometimes can negate each other, limit the possible formulations and methods for preparation of formulations suitable for once-daily administration.

According to the invention, there is provided a pharmaceutical formulation for the once-daily administration of a hydrophilic drug, said formulation comprising biodegradable microcapsules containing at least one low molecular weight hydrophilic drug entrapped in a biodegradable polymer and the release of the or each hydrophilic drug from the microcapsules being substantially diffusion controlled.

The term microcapsule used herein includes the terms "microsphere", "microparticle" and "nanoparticle". These terms do not necessarily refer to any structure relationship between the drug and the encapsulating polymer (e.g., drug encapsulated within a polymer membrane or drug and encapsulating polymer in a matrix structure). Rather, these terms simply refer to a particle (micron sized or less) in which the drug is entrapped in a polymer. The structure of the particle can, however, can be inferred from the release profile of the drug from the particle.

The microcapsules suitably have a D 50% between about 100 nm and 900 nm.

Further, preferably, the hydrophilic drug loading of the microcapsules ranges from about 10% to 70% by weight.

The invention also provides a pharmaceutical formulation for the administration of a hydrophilic drug, said formulation comprising biodegradable microcapsules containing at least one low molecular weight hydrophilic drug entrapped in a biodegradable polymer, said microcapsules having a D 50% between about 100 nm and 900 nm and a drug loading which ranges from about 10% to 70% by weight.

Thus the formulations according to the invention can be used to entrap low molecular weight hydrophilic drugs in the submicron range, which on administration to a patient, in particular, by the oral or rectal route, can provide effective therapeutic amounts over a substantially 24 hour period.

Thus the formulations can be used to administer a low molecular weight hydrophilic drug to a patient on a once-daily basis so as to achieve a therapeutic effect over a substantially 24 hour period.

The release of the or each hydrophilic drug from the microcapsules is preferably substantially diffusion controlled.

The microcapsules suitably have a D 50% between about 200 nm and 400 nm.

Furthermore, the hydrophilic drug loading of the microcapsules preferably ranges from about 20% to 50% by weight.

Preferably the hydrophilic drug is selected from a calcium antagonist, a narcotic analgesic and an ACE-inhibitor and analogues and mixtures thereof.

Suitable calcium antagonists include diltiazem, verapamil, nifedipine, nimodipine and nicardipine.

Suitable narcotic analgesics include hydromorphone, codeine sulfate, oxycodone, dihydrocodeine tartrate, oxycodeinone, morphine, fentanyl, sufentanil, oxymorphone and buprenorphine.

Suitable ACE-inhibitors include captopril, enalapril and lisinopril.

The formulations may also include a combination of two or more hydrophilic drugs as hereinbefore specified. Particularly preferred combinations are represented by a combination of a calcium antagonist and a narcotic analgesic. Synergism is displayed by such combinations. A suitable combination comprises nifedipine and hydromorphone.

The polymer matrix suitably comprises polylactide; polyglycolide; poly(lactic acid-co-glycolic acid); poly(ε-caprolactone); poly(hydroxybutyric acid); polyortho-esters; polyacetals; polydihydropyrans; polycyanoacrylates; polypeptides; cross-linked polypeptides; and steroisomers, racemic mixtures, co-polymers and polymer mixtures thereof.

A particularly suitable polymer matrix comprises poly-L-lactide.

A suitable release profile measured in accordance with the Paddle Method of U.S. Pharmacopoeia XX at 37°C and 75 rpm for the or each hydrophilic drug is substantially as follows:

a) 10-30% release within 2 hours after administration;
b) 30-60% release within 4 hours after administration;
c) 60-80% release within 8 hours after administration; and
d) $\geq$ 80% release within 20 hours after administration.

A further suitable release profile measured in accordance with the Paddle Method of U.S. Pharmacopoeia XX at 37°C and 75 rpm for the or each hydrophilic drug is substantially as follows:

a) 10-40% release within 1 hour after administration;
b) 20-60% release within 4 hours after administration;
c) 40-80% release within 8 hours after administration; and
d) $\geq$ 80% release within 16 hours after administration.

The microcapsules in accordance with the invention are suitably formulated as capsules, tablets, powders capable of effervescing upon addition of water, or suspensions.

Thus, for convenient and effective oral/rectal administration, effective amounts of the microcapsules of the present invention can be tabletted with one or more excipient(s), encased in capsules such as gel capsules, formulated with ingredients which, upon addition of water, provide an effervescent solution, and suspended in a liquid solution and the like. The microcapsules can be suspended in a saline solution or the like for parenteral administration.

It will be appreciated that the pharmaceutical formulations in accordance with the invention can be used *inter alia* in a method of treating angina, hypertension, pain or the like by the administration of a formulation containing biologically effective amounts of at least one low molecular weight hydrophilic drug microencapsulated according to the invention to an animal, preferably a human, so as to provide and maintain clinically effective plasma levels of the drug throughout a substantial part of a 24 hour period.

The invention also provides a method for the manufacture of microcapsules as hereinbefore defined, which comprises the steps of:

a) dissolving or dispersing a low molecular weight hydrophilic drug and a biodegradable polymer in a solvent to form a mixture;

b) microfluidising said mixture into an external phase to form an emulsion in which the emulsion droplets have a mean diameter less than 1 μm; and

c) stirring said emulsion to form microcapsules having a size (D 50%) between about 100 nm and 900 nm.

As used herein, the term "biodegradable" as applied to polymers means synthetic or natural polymers which are degradable *in vivo* either enzymatically or non-enzymatically to produce biocompatible or non-toxic by-products which can be further metabolised or excreted *via* normal physiological pathways. For instance, a range of natural and synthetic biodegradable polymers can be used to form microparticles containing a wide variety of drugs in order to achieve prolonged drug release or drug targeting. Human serum albumin (HSA), bovine serum albumin (BSA), collagen and gelatin can be used to microencapsulate the drugs. However, the cost and uncertainty of purity of natural polymers limits their use, thus focusing attention upon synthetic biodegradable polymers in which processing conditions and availability can be controlled. Examples of synthetic biodegradable polymers are those hereinbefore specified and include the following: polylactic acid (polylactide; PLA); polyglycolic acid (polyglycolide; PGA); poly(lactic acid-co-glycolic acid) (PLGA); poly(ε-carpolactone); poly(hydroxybutyric acid); polyortho-esters; polyacetals; polydihydropyrans; polycyanoacrylates; polypeptides; cross-linked polypeptides; and steroisomers (i.e., D, L), racemic mixtures, co-polymers and polymer mixtures thereof. PLA is the biodegradable polymer which is most preferred.

The solvent evaporation method is one of the most common techniques for the production of PLA/PLGA microspheres. The first step in this method requires the formation of an oil-in-water emulsion (o/w emulsion). The polymer is dissolved in a volatile organic solvent followed by dissolution or dispersion of the drug in the polymer solution. This procedure is known as forming the organic phase. The resulting polymer/drug solution is stirred in a suitable suspension medium forming a droplet suspension (o/w emulsion). The suspension medium, which should not solubilise the polymer, usually contains a dispersing agent to prevent coalescence of the droplets. The suspension

medium may also be referred to as the continuous phase or the external aqueous phase, the latter term being used herein.

A modification of this solvent extraction method can be used wherein the polymer and drug are first dissolved in a water miscible organic solvent such as acetonitrile and subsequently homogenised in mineral oil or light liquid paraffin to form a "w"-in-oil ("w"/o) emulsion. Another modification of this method is used, whereby a water-oil-water (w/o/w) emulsion is produced. The drug is dissolved in a small amount of water and this drug solution homogenised into a polymer/organic solvent (e.g. dichloromethane) solution. This primary water-in-oil emulsion is then emulsified with the external phase, producing the w/o/w emulsion.

Once the emulsion is formed (o/w, w/o, or w/o/w), it is subjected to solvent evaporation causing 'hardening' of the droplets (polymer solidification) and their conversion to microspheres. The emulsion is stirred continually throughout the evaporation process. During the droplet hardening process, the droplets first lose their solvent by diffusion of the solvent into the external aqueous phase followed by evaporation of the solvent from the surface. The evaporation of the solvent can take place at either atmospheric pressure or at reduced pressure. The evaporation temperature can also be varied. Solvent evaporation can be achieved in two ways: (1) interrupted evaporation in which the evaporation is stopped prior to complete elimination of the organic solvent, and the semi-solid microspheres are transferred to an emulsifier-free medium where evaporation is completed or (2) continuous evaporation in which the system is continuously agitated until all the organic solvent has completely evaporated. The solid microspheres are collected by filtration or centrifugation, washed and dried under vacuum.

Problems associated with the solvent evaporation method are agglomeration of the particles, loss of drug to the aqueous phase and the formation of drug crystals on particle surfaces. Factors affecting the quality of microspheres (i.e., drug loading, size, surface

morphology), produced by the solvent evaporation method are detailed below.

In the solvent evaporation process, the organic solvent used, in addition to being able to dissolve the drug and the polymer, must be immiscible with the external phase and its boiling point must be low enough so that it will evaporate easily during the droplet hardening process. When an o/w system is used, the solvent evaporation technique works best for core materials that are insoluble in the aqueous external phase. If the core material is soluble in the external phase, it will be extracted from the microdroplets before the microsphere walls have a chance to form, resulting in lower drug loading. For instance, Bodmeier and McGinity, J. Microencap., *4*:279-288 (1987) reported that water soluble drugs such as theophylline, caffeine and salicylic acid could not be entrapped by the o/w emulsion solvent evaporation method. Minimising drug loss to the aqueous external phase can be achieved in a number of ways, such as (1) by varying the aqueous phase pH to the pH of minimal drug solubility; (2) saturating the aqueous phase with the drug; and (3) inverting the emulsification phase o/w to w/o.

The effect of using different molecular weights for a particular polymer affects the surface properties and morphology of the microspheres. For instance, high molecular weight poly-L-lactide polymers produce larger particles that tend to aggregate due to the inherent tackiness and thermoplastic nature of the polymer. Also, highly porous microspheres are produced from high molecular weight polymers whereas microspheres produced with low molecular weight polymers can be smooth and non-porous.

The diameter of the microspheres can be determined by the size of the microdroplets formed in the emulsion. The size of the microdroplets can be controlled in several ways, such as by adjusting the stirring rate, the type and the amount of emulsifier, the quantity of the aqueous phase, and the configuration of the vessel and stirrer. A general increased rate of agitation results in a finer emulsion producing

smaller microspheres and preventing coalescence of smaller immature microspheres

We have found that very fine emulsion droplets can be prepared by using a Microfluidiser (Trade Mark; Microfluidics Corp.) A Microfluidiser is a high pressure homogeniser capable of making very small size emulsion droplets (mean droplet diameter under 1 μm). During microfluidisation, the emulsion is pumped through microchannels to an impingement area at high operating pressures. Cavitation and the accompanying shear and impact is responsible for the particle size reduction within the "interaction chamber".

Drug release from biodegradable systems may range from purely diffusion-controlled dissolution from a matrix system in cases in which the polymer has a slow degradation rate and the drug molecule is small, to degradation-controlled release when the drug molecule is large and its permeability in the polymer is low. With the more rapidly degrading polymers, drug release can occur through a combination of diffusion and degradation of the polymer. The possible mechanisms of drug release from biodegradable microsphere systems may be summarised as follows: (1) drug which is loosely bound to or embedded in the microsphere surface is released initially resulting in a "burst effect"; (2) depending on microsphere structure, drug may be released through the microsphere pores; (3) drug may diffuse through the polymer membrane depending on the solubility of the drug in the polymer membrane and the intrinsic properties of the polymer; (4) the drug may diffuse through the water swollen polymer barrier depending on the hydrophilicity and in turn the molecular weight of the polymer; and (5) erosion and hydrolysis of the polymer chains causes pore formation in the microsphere structure and hence affects the rate of drug release. All these possible mechanisms may together play a part in the release process depending on the morphology of the microsphere systems (which in turn is affected by manufacturing techniques), polymer composition and physiochemical properties of the drug.

Drug release from microsphere systems is dependent on the diffusivity of the drug through the polymer barrier, the solubility of the drug in the bulk phase, the size of the drug molecule and its distribution throughout the microsphere matrix. The nature of the polymer plays a major role in determining release characteristics, in particular the molecular weight of the polymer. For instance, the effect of polymer molecular weight on *in vitro* release of chlorpromazine from chlorpromazine loaded poly-DL-lactide microspheres using poly-DL-lactide in a molecular weight range from 11,000 to 21,000, in pH 7.0 aqueous phosphate buffer has been studied. Increased polymer molecular weight increased the time required for 50% release. Microspheres produced from the higher molecular weight polymers exhibited a lag time of $\geq$ 24 hours at which time the release rate increased rapidly to approximately $\leq$ 40% release.

Furthermore, two of the most important microsphere characteristics that affect drug release are drug loading and microsphere size.

Early theories which have been put forward to describe the dissolution process from matrix systems were very often based on Fick's laws of diffusion. Fick's first law of diffusion states that the flux (J) of a compound across a unit area of a predetermined reference plane is proportional to the concentration differential across that plane, the proportionality constant being the diffusivity (D). Based on Fick's first law of diffusion, Higuchi developed an equation for release of solid drug from an ointment base and later applied it to the diffusion of solid drugs dispersed in homogenous and granular matrix dosage systems as follows:

$$Q = (D(2A-Cs)\ Cs\ T)^{\frac{1}{2}} \qquad \text{(Equation 1)}$$

where D = the diffusivity of the drug in the matrix;
A = the total amount of drug per unit volume of matrix;
Cs = the solubility of drug in the polymer matrix;
Q = amount of drug per unit area of matrix; and

T = time.

Later, using the same diffusion principle as Higuchi, Cobby *et al.* presented equations describing the release of drug from matrix tablets having either a spherical, cylindrical or biconvex shape. In each case, the three dimensional equation had the cubic form:

$$F = G_1(KT^{\frac{1}{2}}) - G_2(KT^{\frac{1}{2}})^2 + G_3(KT^{\frac{1}{2}})^3 \qquad \text{(Equation 2)}$$

where F = the fraction of drug released;
T = the elapsed time;
K = the release rate constant;
G_1 - G_3 are shape factors.
For a spherical shape G_1 and $G_2 = 3$ and $G_3 = 1$.

The fundamental parameters of the release rate constant are described by

$$K = 1/A \,.\, r \sqrt{}\, D\, C_S\, (2A-\varepsilon C_S)\, \varepsilon/\tau.$$

where r = initial tablet radius;
ε = porosity; and
τ = tortuosity.

The Prout-Tomkins' equation has been applied to the autocatalytic decomposition of solid, i.e., the drug acting as a marker of polymer degradation:

$$\ln (\chi/1-\chi) = K \times t + c \qquad \text{(Equation 3)}$$

where χ = the fraction of drug released at time t;
K = the rate constant; and
$c = K \times t_{max}$ (t_{max} is the time taken for 50% of the drug to be released).

The release of diltiazem base from DL-PGLA microspheres in phosphate buffer pH 7.4 has been fitted to the Prout-Tomkins' equation (Fitzgerald *et al.*, Polymeric Delivery Systems, Properties and Applications, Chap. 23 (ACS Symposium Series 520) (1992)). The systems studied had drug loadings of 4.45%, 10.06% and 20.22 % and were in the <125 μm size range. In all cases, the fits obtained were good, suggesting that release from these systems was dependent on polymer degradation and dissolution.

Various known methods can be used to obtain microcapsules as follows.

Drug Dissolved in Solvent Method (Method A)

This method is similar to the oil-in-water (o/w) emulsion solvent evaporation techniques. The drug and polymer are dissolved in a suitable solvent (such as dichloromethane) to form the oil phase. An aqueous 0.27% w/v PVA solution is suitably used as the external phase. The oil phase is emulsified into the external phase to form a droplet suspension for example by one of the following methods:

(i) Using an IKA Ultra Turrax T25 (Trade Mark) for two minutes at 8,000, 9,500, 13,500 or 24,000 rpm;

(ii) Using an M120E Microfluidiser (Microfluidics Corp.) at 10,000 psi for a specified number of cycles.

The resulting emulsion can be stirred for example with an IKA RW25 motor and 4-blade stirrer at 1,400 rpm for one hour causing the polymer droplets to lose their solvent, initially by diffusion of the solvent into the aqueous external phase and subsequently by evaporation. In this way, the polymer droplets are converted to the corresponding solid microspheres.

The microspheres are suitably collected by centrifugation (Heraeus Sepatech Biofuge 28RS) using a HFA 13.5 rotor at 13,000

rpm for 15 minutes and then dried in a vacuum oven (Gallenkamp) at 1,000 mbar for 24 hours. The microspheres are stored in airtight containers at room temperature.

Dispersion Method (Method B)

The oil phase is formed by dispersing the drug in the polymer/solvent solution using sonication. The oil phase is emulsified into the external aqueous phase by Method A(i) or (ii). This step is followed by solvent evaporation, collection, drying and storage as outlined in Method A. Final microsphere size is dependent on drug crystal size. This limitation can be overcome by dissolving the drug in a co-solvent as outlined in Method C below.

Co-Solvent Method (Method C).

The drug is first dissolved in the minimum amount of a suitable solvent (such as methanol). The polymer/solvent solution is then added to the drug solution and sonicated to form the oil phase. The oil phase is emulsified into the external aqueous phase by Method A(i) or (ii). This step is followed by solvent evaporation, collection, drying and storage as outlined in Method A. As described below, diltiazem HCl was entrapped using Method C in microspheres formed from PLA with a molecular weight average of 109,000 (PLA 109K). The formulation used for a 10% w/w starting loading was as follows:

PLA 109K : Dichloromethane	0.9 g : 10 ml
Diltiazem HCl : Methanol	0.1 g : 0.5 ml
Dichloromethane : 0.27% PVA	1 ml : 10 ml

For all methods when entrapping diltiazem, the PVA solution is suitably buffered to pH 10.0 (pH of least solubility of diltiazem in an aqueous solution) suitably using a carbonate buffer prepared as follows:

$NaHCO_3$	3.915 g/l
Na_2CO_3	5.660 g/l

Double Emulsion Method (Method D)

This method is an adaptation of a (water-in-oil)-in-water (w/o/w) solvent evaporation technique which has previously been found to be successful in entrapping hydrophilic agents in polylactide-co-glycolide microparticles (Ogawa *et al.*, Chem. Pharm. Bull., *36*:1502-1507 (1988)). In a modification of this method carried out by us the drug was dissolved in a 5% w/v gelatin solution (internal aqueous phase). The polymer/dichloromethane solution was added to the drug solution and emulsified at 24,000 rpm for one minute using an IKA Ultra Turrax T10. The resulting w/o emulsion was then emulsified into the 0.27% w/v PVA external aqueous phase by one of the following methods to produce the w/o/w emulsions:

(i) Using an IKA Ultra Turrax T18 at 8,000 rpm for two minutes;

(ii) Using an M120E Microfluidiser (Microfluidics Corp.) at 10,000 psi for a specified number of cycles.

The droplet hardening process was carried out by stirring the w/o/w emulsion at 1,400 rpm for 30 minutes - 1 hour (depending on the amount of solvent used) with an IKA RW25 and 4-blade stirrer. The microspheres were collected, dried and stored as outlined for Method A. For diltiazem HCl, the drug: gelatin solution ratio was 0.1 g : 0.1 ml; the polymer : solvent ratio was PLA 109K 1 g : 4 ml; and the external phase was 100 ml per 2 g total batch weight.

***In vitro* Dissolution Studies**

Dissolution studies herein were carried out in the following dissolution media:

(i) Isotonic phosphate buffer pH 7.4

NaCl	4.4 g/l
NaH_2PO_4 (anhydrous)	1.615 g/l
Na_2HPO_4 (anhydrous)	7.571 g/l

(ii) Simulated gastrointestinal fluid (SGF) pH 1.2

NaCl	2.0 g/l
Concentrated HCl	7.0 ml/l

Stoppered dissolution flasks were filled with a measured volume of the desired dissolution medium and placed in a water bath at a constant temperature of 37°C. After the solution had reached equilibrium, a desired amount of sample was added into the flask. The sample weight was predetermined to prevent the drug concentration in the medium from exceeding 10% of its solubility and thus maintain sink conditions. The samples were either dispersed in the dissolution media, or, if the samples were in the submicron size range, they were contained in a 14 cm length of Visking tubing tied at both ends. The dissolution flasks were shaken at 60 spm.

In the accompanying drawings:

Fig. 1 shows the release profiles in phosphate buffer pH 7.4 of diltiazem HCl from 10% w/w loaded PLA 109K microsphere systems prepared by Method C, D_1 (D 50% of 12.56 μm), D_2 (D 50% of 5.76 μm); D_3 (D 50% of 2.86 μm); and D_4 (D 50% of 1.41 μm);

Fig. 2 shows the release profiles in SGF pH 1.2 of diltiazem HCl from 10% w/w loaded PLA 109K microsphere systems prepared by Method C, D_1 (D 50% of 12.56 μm), D_2 (D 50% of 5.76 μm); D_3 (D 50% of 2.86 μm); and D_4 (D 50% of 1.41 μm);

Fig. 3 shows release profiles in phosphate buffer pH 7.4 of diltiazem base from PLA 109K microsphere systems prepared by Method A with the Ultra Turrax (Db_2-Db_5) 10%, 20%, 30% and 50% w/w, respectively;

Fig. 4 shows release profiles in SGF pH 1.2 of diltiazem base from PLA 109K microsphere systems prepared by Method A with the Ultra Turrax (Db_2-Db_5, 10-50% w/w respectively); and

Figs. 5, 6 and 7 show, respectively, release from 20%, 30% and 50% diltiazem base loaded PLA 109K nanospheres at pH 1.2, pH 6.0 and pH 7.4 (as prepared in Example 8).

Conversion of Diltiazem HCl to Diltiazem Base

Diltiazem HCl was converted to the base form when required herein by preparing a saturated diltiazem HCl aqueous solution and adding an excess of 1M NaOH until precipitation occurred. Recovery of the diltiazem base was followed by drying in a desiccator under vacuum for two days.

The invention will be further illustrated by the following Examples.

EXAMPLE 1

An attempt was made to prepare diltiazem loaded PLA microspheres using Method A, a method involving as described above the evaporation of solvent from an o/w emulsion, the oil phase being formed by dissolving the drug and polymer in dichloromethane. This method was not suitable as diltiazem HCl did not dissolve in dichloromethane.

EXAMPLE 2

Attempts to prepare diltiazem HCl loaded PLA microspheres by Method B were unsuccessful. Method B also involved the evaporation of solvent from an o/w emulsion, the oil phase being formed by dispersing the drug in the polymer/solvent solution using sonication. Diltiazem HCl crystals when observed under the light microscope were seen to be too large and irregular in shape for entrapment by this method. This finding was substantiated by particle size analysis: diltiazem HCl had a D 10% of 19.01 μm, D 50% of 41.83 μm and a D 90% of 72.61 μm.

EXAMPLE 3

Diltiazem HCl loaded PLA 109K microspheres were prepared using Method C, whereby the drug was first dissolved in the minimum amount of a suitable solvent (methanol). The polymer/solvent solution was then added to the drug solution and sonicated to form the oil phase. The oil phase was emulsified into the external aqueous phase (buffered to pH 10.0) using the IKA Ultra Turrax at four different homogenisation speeds. All the systems prepared by Method C had a starting loading of 10% w/w. Microsphere system D_1 was prepared at the lowest homogenisation speed setting on the Ultra Turrax, i.e., 8,000 rpm., the microspheres produced had a drug entrapment efficiency of 86.0%. The homogenisation was subsequently increased to 9,500, 13,500 and 24,000 rpm to produce microsphere systems D_2, D_3 and D_4 having drug entrapment efficiencies of 98.7%, 89.4% and 63.8% respectively, as shown in Table 1.

Table 1

Lot No.	Speed of Homogenisation Ultra Turrax rpm	Entrapment efficiency %	Yield %	D 10% µm	D 50% µm	D 90% µm	Span
D_1	8000	86.0	80.5	3.76	12.56	67.61	4.97
D_2	9500	98.7	80.0	2.29	5.76	16.39	2.44
D_3	13500	89.4	86.0	0.67	2.86	5.61	1.73
D_4	24000	63.8	79.5	0.65	1.41	3.59	2.09

There was a marked decrease in the size of the microspheres with increasing homogenisation speed. The D 50%, of the microspheres decreased from 12.85 µm to 1.41 µm as the homogenisation speed was increased from 8,000 rpm to 24,000 rpm. There was a corresponding decrease in the D 90% from 67.61 µm to 3.59 µm and in the D 10% from 3.76 µm to 0.65 µm. This relationship was not linear when plotted on a log-log scale.

The entrapment efficiency of the microspheres was found to be dependent on the homogenisation speed used. With increasing homogenisation speed, there was an initial slight increase followed by a drop in efficiency with further decrease in size. This may be due to the fact that the smaller particles have a larger surface area/volume ratio resulting in greater drug loss to the aqueous external phase during processing. Percentage entrapment efficiency ranged from 63.8% to 98.7%. It was not possible to exceed 10% w/w starting loading as the amount of diltiazem needed for a starting loading greater than 10% w/w would not dissolve in 0.5 ml of methanol and in this formulation the ratio of methanol to dichloromethane should not exceed 0.5 ml to 10 ml.

SEM photomicrographs of all the microsphere systems prepared by Method C show smooth, spherical microspheres. Microspheres produced with the lowest homogenisation speed 8,000 rpm were large with a D 50% value of 12.56 μm and had a wide size distribution, as indicated by the span value of 4.97. In comparison, microspheres produced with the highest homogenisation speed, 24,000 rpm, were smaller with a D 50% value of 1.41 μm and had a narrower size distribution, as indicated by the span value of 2.09. Microspheres produced at 9,500 and 13,000 rpm were also more uniform in size than D_1 with span values of 2.44 and 1.73, respectively.

The diltiazem HCl loaded PLA 109K microspheres prepared by Method C were analysed by differential scanning calorimetry (DSC). The diltiazem HCl used was crystalline having a thermal event at 217°C. PLA 109K showed a thermal event at 65.6°C. DSC thermograms of the microspheres prepared by Method C showed no detectable crystalline diltiazem HCl. Physical mixtures containing 10% w/w and 5% w/w diltiazem HCl in PLA 109K had thermal events at 215.2°C, 65.5°C and 214.0°C, 63.9°C, respectively. A DSC scan of a 3% diltiazem in PLA 109K physical mixture showed a thermal event at 65.9°C only, indicating that 3-5% is the limit of detection for a diltiazem HCl/PLA 109K system.

X-ray diffraction patterns show the absence of crystalline diltiazem HCl in the systems prepared by Method C, indicating that diltiazem HCl is present in an amorphous or solubilised state.

EXAMPLE 4

Diltiazem HCl loaded PLA 109K microsphere systems were prepared by Method D, a method involving the evaporation of solvent from a double emulsion (w/o/w emulsion). The drug was initially dissolved in a 5% w/v gelatin solution to form the internal aqueous phase. The polymer/dichloromethane solution was added to the drug solution and emulsified using the Ultra Turrax at 24,000 rpm for one minute. The resulting w/o emulsion was then emulsified into the

external aqueous phase (buffered to pH 10.0) using the Ultra Turrax at 8,000 rpm for two minutes. Microspheres with a starting loading of 10% w/w (D_5) were produced and had an entrapment efficiency of 96.8%. The starting loading was subsequently increased to 20% w/w (D_6); the microspheres produced had an entrapment efficiency of 85.5%, finally, microspheres with a starting loading of 30% w/w (D_7) had an entrapment efficiency of 71.5%. As the starting loading was increased from 10% w/w to 30% w/w the entrapment efficiency decreased from 96.8% to 71.5%.

Using Method D, it was possible to prepare microspheres with up to 30% w/w starting loading, whereas with Method C it was not possible to exceed a starting loading of 10% w/w due to the limited solubility of the diltiazem HCl in the co-solvent (methanol). For Method D, in order to control the viscosity of the primary emulsion, the drug to gelatin ratio was kept at 0.1 g : 0.1 ml. Therefore, it was not possible to exceed a starting loading of 30% w/w as the diltiazem HCl necessary to achieve a drug loading of greater than 30% w/w would not dissolve in the gelatin solution.

Method D resulted in batches with lower percentage yield (54.0 - 63.5%) than those obtained using Method C (79.5 - 86.0%). This may be due to greater loss of product to vessel surfaces and homogeniser heads etc. during the two step process involved in Method D. Entrapment efficiency results shown in Table 2, for Methods C and D were comparable, ranging from 63.8-98.7% for Method C to 71.5-96.8% for Method D.

TABLE 2

Lot No.	Starting Loading % w/w	Actual Loading % w/w	Entrapment efficiency %	Yield %	D10% µm	D50% µm	D90% µm	Span
D_5	10	9.68	96.8	60.0	1.37	5.39	15.81	2.65
D_6	20	17.10	85.5	54.0	3.34	15.95	42.97	2.60
D_7	30	21.46	71.5	63.5	2.88	9.43	60.65	4.39

SEM photomicrographs of the systems produced by Method D showed the particles to be spherical, however the surfaces of these particles showed some irregularities. System D_7, 30% w/w starting loading had some drug crystals present between the microspheres and on the microsphere surfaces. Particle size distribution plots for microsphere system D_5 showed bimodal distribution of particles whereas particle size distribution plots for microsphere system D_7 show multimodal distribution, which may be due to the presence of drug crystals, polymer debris or very small particles. Microsphere size was found to increase with increased drug loading. Microsphere system D_5, 10% w/w starting loading, had a D10% of 1.37 µm and a D90% of 15.81 µm compared to a D10% of 2.99 µm and a D90% of 60.65 µm for (D_5), 30% w/w starting loading.

EXAMPLE 5

In-Vitro Release Studies: The effect of particle size on the release of diltiazem HCl from 10% loaded PLA 109K microspheres

Prior to carrying out *in vitro* release studies, the saturated solubility of diltiazem HCl was measured in order to establish sink conditions. Diltiazem HCl was found to have Cs values of 2.39 g/l in phosphate buffer pH 7.4, and greater than 1 g/ml in pH 1.2. The microsphere systems studied had a diltiazem HCl loading of 10% w/w and were prepared by Method C.

Release profiles of diltiazem HCl from PLA 109K microspheres obtained in isotonic phosphate buffer pH 7.4 showed release to be retarded when compared with release of pure diltiazem HCl. Secondly, release was found to be related to the size of the particles. For a given mass of particles, the larger size microsphere released diltiazem at a slower rate than the smaller size particles. The release profiles are in rank order from the largest microspheres, D_1 produced at 8,000 rpm with a D50% of 12.56 μm, to D_4 produced at 24,000 rpm with a D50% of 1.41 μm (Fig. 1). Release profiles of diltiazem HCl from PLA 109K microspheres obtained in SGF pH 1.2 also showed release to be retarded when compared with release of pure diltiazem HC1. In SGF, there was a high initial "burst" effect, smaller particles released up to 60% of drug in the first 24 hours. This was probably due to the solubility of the drug being greater in SGF than in phosphate buffer, The release profiles of the larger microsphere systems D_1 (D50% of 12.56 μm, to D_4 produced at 24,000 rpm with a D50% of 1.41 rpm.

Release profiles of diltiazem HCl from PLA 109K microspheres obtained in SGF pH 1.2 also showed release to be retarded when compared with release of pure diltiazem HCl. In SGF, there was a high initial "burst" effect, smaller particles released up to 60% of drug in the first 24 hours. This was probably due to the solubility of the drug being greater in SGF than in phosphate buffer. The release profiles of the larger microsphere system D_1 (D50% of 12.56 μm) and D_2 (D50%

of 5.76 μm) were similar: drug release from these systems was very slow. The smaller systems D_3 (D50% of 2.86 μm) and D_4 (D50% of 1.41 μm) also had similar release profiles and released diltiazem at a faster rate (Fig. 2).

Release profiles were fitted to Equation 2, which describes the release of drug from a spherical matrix. The rate constant K is defined by the Higuchi type equation. The best estimates of K by non-linear least square and values of correlation coefficient are given below in Table 3. The fits were considered relatively good in phosphate buffer pH 7.4, as measured by the correlation coefficients. Release appeared to be primarily diffusion controlled.

TABLE 3

Lot No.	D 50% μm	K	correlation
D_1	12.56	0.00382	0.099714
D_2	4.76	0.01008	0.97049
D_3	2.86	0.01782	0.99170
D_4	1.41	0.29023	0.99608

The correlation coefficients of the release data in pH 1.2 were poor. When the release data for the first five hours of diltiazem release in SGF was fitted to Equation 2, the correlation coefficients were much improved, indicating that release at the earlier time points was diffusion controlled. Values of K were found to be inversely proportional to particle size. As the D 50% decreased from 12.85 μm (D_1) to 1.41 μm (D_4), the value of K increased from 0.00382 to 0.29023 in phosphate buffer, and from 0.00783 to 0.03586 for release in the first five hours in SGF.

Release studies were stopped after 500 hours. The microspheres were collected, dried and assayed after dissolution in SGF. The drug

content of the microspheres was as follows D_1:6.70% w/w; D_2: 7.42% w/w; D_3 3.06% w/w; D_4: 2.35% w/w, i.e., the drug had not degraded in the microspheres. Subsequent release from these systems may have been controlled by degradation of the polymer.

EXAMPLE 6

Diltiazem base is soluble in dichloromethane, therefore Method A, a method involving the evaporation of solvent from an o/w emulsion, the oil phase being formed by dissolving the drug and polymer in dichloromethane was suitable for the preparation of diltiazem base loaded PLA 109K microspheres. All batches were prepared using a 1:11 ratio of PLA 109K dichloromethane. Microspheres with a starting loading of 10% w/w up to 80% w/w were prepared. The oil phase was homogenised into the external aqueous phase (buffered to pH 10.0) using the IKA Ultra Turrax at 8,000 rpm for all systems except Db_1. As shown in Table 4, entrapment efficiencies in the range 61.85 - 88.5% were achieved. Product yield was high at all loadings, ranging from 83.0% to 97.0%.

TABLE 4

Lot No.	Starting Loading % w/w	Actual Loading % w/w	Entrapment efficiency %	Yield %	D10% µm	D50% µm	D90% µm	Span
Db_1	10	6.99	69.9	84.5	2.62	18.58	76.72	4.00
Db_2	10	7.63	76.3	83.0	1.21	3.27	7.17	1.82
Db_3	20	16.61	83.04	97.0	1.51	4.02	7.25	1.43
Db_4	30	18.53	61.8	87.5	1.19	3.45	6.10	1.42
Db_5	50	36.27	72.53	88.0	1.27	4.02	7.72	1.61
Db_6	50	33.43	66.86	95.5	1.69	5.86	8.90	1.23
Db_7	80	70.78	88.5	86.3	2.75	13.07	27.03	1.23
Db_8	90	not possible	-	-	-	-	-	-

Using a 10% w/w starting loading, microsphere system Db_2 was prepared using the IKA Ultra Turrax at the lowest speed setting of 8,000 rpm to emulsify the oil phase into the external equeous phase. Microspheres with a D50% of 3.27 µm were produced (diltiazem HCl loaded microspheres prepared at the same homogenisation speed using Method C had a larger D50% of 12.56 µm).

Microsphere system Db_1 was prepared using a T25 stirrer at a much lower speed setting of 1,400 rpm to emulsify the oil phase into

the external aqueous phase. The microspheres were larger as expected with a D50% of 18.58 μm. The microspheres prepared with the IKA Ultra Turrax had a higher entrapment efficiency than those prepared with the T25 stirrer. Since it was established that the IKA Ultra Turrax could efficiently produce small particles at 8,000 rpm using Method A, this speed setting was used for subsequent batches Db_3 -Db_7.

Diltiazem base is less soluble in water than diltiazem HCl, therefore significantly higher drug loading could be achieved (up to 80% w/w starting loading) as there was very little loss of drug to the external aqueous phase. It was not possible to exceed a starting loading of 80% w/w as the amount of diltiazem base necessary to achieve greater loading would not dissolve in the given amount of dichloromethane. Using Method A with the Ultra Turrax, microspheres with entrapment efficiencies in the range 61.8-88.5% were produced. Entrapment efficiency was independent of starting loading. Product yield ranged from 83.0% to 97.0% and was also independent of starting loading.

Diltiazem base loaded PLA 109K microsphere systems prepared by Method A at drug loadings of 10, 20, 50 and 80% w/w were analysed by DSC. The diltiazem base starting material used had a thermal event at 105°C. PLA 109K had a thermal event at 65.6°C. Physical mixtures containing 10% and 30% w/w diltiazem base had thermal events at 64.8°C, 104.4°C, 64.6°C and 104.7°C, respectively. The limit of detection for a diltiazem base/PLA 109K system is 10% w/w. DSC thermograms of systems Db_2, Db_3 and Db_5 showed no detectable crystalline diltiazem base, whereas Db_7 had a thermal event at 105.4°C, indicating the presence of crystalline diltiazem base.

SEM photomicrographs of diltiazem base loaded PLA 109K systems prepared by Method A using the Ultra Turrax showed spherical particles. The surfaces of the particles were smooth. However, at 50% w/w loading there was some debris on the microsphere surfaces and in between the microspheres; this may be

polymer or drug crystals. At 80% w/w loading there was a considerable amount of drug crystals present.

With the exception of Db_7, particle size results of all microsphere systems prepared with the Ultra Turrax were very reproducible, with D10% ranging from 1.19-1.69 μm, D50% ranging from 3.27-5.86 μm and D90% ranging from 6.10-8.90 μm. Microspheres of system Db_7 were larger with a D 50% of 13.07 μm. Microsphere systems prepared with the Ultra Turrax (Db_2 - Db_6) were also reasonably uniform in size with span values ranging from 1.23-1.82. In contrast, microspheres of microsphere system Db_1, prepared at 1,400 rpm with the T25 stirrer, were much larger (D50% of 18.58 μm) and had a wider size distribution, as indicated by a span value of 4.00 and particle size distribution plots.

EXAMPLE 7

In-vitro release studies: The effect of drug loading on the release of diltiaxem base from PLA 109K microspheres

Prior to carrying out *in vitro* release studies, the saturated solubility of diltiazem base was measured in order to establish sink conditions. Diltiazem base was found to have Cs values of 227.47 g/l in SGF pH 1.2 and 1.06 g/l in phosphate buffer pH 7.4. The microsphere systems studied had diltaizem base starting loadings of 10% w/w, 20% w/w, 30% w/w, and 50% w/w, D 50% of 3.27 μm, 3.45 μm, 4.02 μm, respectively, and were prepared by Method A using the Ultra Turrax.

Release profiles of diltaizem base from PLA 109K microspheres in isotonic phosphate buffer pH 7.4 showed release to be dependent on percentage drug loading. Microspheres at the lower drug loading of 10% w/w released diltiazem base at a slower rate. The release profiles were in rank order the lowest drug loading 10% w/w to the highest drug loading 50% w/w. There was very little difference in the release profiles of microspheres at 30% and 50% w/w (Fig. 3). Release profiles of diltiazem base from PLA 109K microspheres in SGF pH 1.2

also showed release to be dependent on percentage drug loading. The release profiles were again in rank order (Fig. 4). There was a notable difference between the release profiles of microspheres at 30% and 50% w/w loading. Microspheres at 50% w/w loading released diltiazem base at a very fast rate. Diltiazem base had a high solubility in SFG. Additionally, at high drug loadings there may be drug at or near to the surface of the microspheres; this may explain the very fast release rate from microspheres at 50% w/w loading. The release profiles in phosphate buffer and in SGF were fitted to Equation 2.

The fits may be considered relatively good, as measured by the correlation coefficients. Release in phosphate buffer appeared to be primarily diffusion controlled. Release in SGF from microspheres with high drug loading also appeared to be primarily diffusion controlled. At lower drug loadings (20% w/w), release was diffusion controlled at the earlier time points. Later release may have been controlled by a combination of diffusion and polymer degradation as indicated by SEM photomicrographs after dissolution. Values of K were found to be proportional to A, the mass of drug initially present. As the starting drug loading increased from 10% w/w (Db_2) to 50% w/w (Db_5), the value of K increased from 0.00378 to 0.02130 in phosphate buffer and from 0.00229 to 0.22015 in SGF.

SEM phomicrographs taken after dissolution of microsphere system Db_3, 20% w/w drug loading, showed complete degradation of the microspheres in both phosphate buffer and SGF suggesting that release at later time points was controlled by a combination of diffusion of the drug into the dissolution medium and degradation of the polymer. Microspheres at 50% w/w drug loading, Db_5 remained intact in SGF, suggesting that at higher drug loading release in SGF was totally diffusion dependent due to the high solubility of the drug in SGF. These microspheres were degraded in phosphate buffer as release in phosphate buffer was slower due to the reduced solubility of the drug and the faster degradation of the polymer at pH 7.4.

EXAMPLE 8

Diltiazem base loaded PLA 109K microspheres were prepared by Method A using the Microfluidiser (Trade Mark), a high pressure homogeniser, to homogenise the oil phase into the external aqueous phase (buffered to pH 10.0). The Microfluidiser was operated at 10,000 psi for one cycle. All batches were prepared using a 1:11 ratio of PLA 109K : dichloromethane. As shown in Table 5, microspheres with a starting loading of 10% w/w were prepared and an entrapment efficiency of 53.7% was achieved. Subsequent systems with starting loadings of 20%, 30% and 50% w/w were prepared. Entrapment efficiencies of 68.1%, 65.2% and 84.5% respectively were achieved and product yields in the range of 56.0% to 67.5% were obtained.

TABLE 5

Lot No.	Starting loading % w/w	Actual loading % w/w	Entrapment efficiency %	% yield	Z average nm
Db_9	10	5.37	53.7	67.0	302.5
Db_{10}	20	13.61	68.1	60.0	294.0
Db_{11}	30	19.56	65.2	67.5	307.5
Db_{12}	50	42.08	84.2	56.0	310.0

Whether the Ultra Turrax or the Microfluidiser was used for homogenisation, the range of entrapment efficiency was not affected; the Ultra Turrax produced batches with entrapment efficiencies in the range of 61.8-88.5% while the Microfluidiser produced batches with entrapment efficiencies in the range of 53.7-84.2%. However, the percentage yields were lower when the Microfluidiser was used for homogenisation. That is, yields of 56.0-67.0% were achieved with the Microfluidiser compared to 83.0-97.0% with the Ultra Turrax. This

may be due to the difficulty in fully recovering product from the Microfluidiser.

Microsphere systems prepared by Method A using the Microfluidiser, Db_9-Db_{12}, had Z average mean size values in the range of 294-310 nm, illustrating that the Microfluidiser is a very effective homogeniser capable of producing very fine emulsion droplets. The particle size was independent of drug loading. Systems with a 10% w/w loading had a Z average mean size value of 302.5 nm while systems with 50% w/w loading had a Z average mean size value of 310.0 nm. The particles produced by this method had smooth surfaces and were spherical in shape. These systems were also uniform in size as seen in SEM photomicrographs of the systems.

EXAMPLE 9

In vitro release studies: The effect of drug loading on the release of diltiazem base from PLA 109K microspheres prepared with the Microfluidiser

The microsphere systems studied had diltiazem base starting loadings of 10% w/w, 20% w/w, 30% w/w and 50% w/w, Z average mean sizes of diameter (D50%) 302.5 nm, 294.0 nm, 307.5 nm, and 310.0 nm, respectively, and were prepared by Method A using the Microfluidiser. Release profiles of diltiazem base from PLA 109K microspheres, obtained in isotonic phosphate buffer pH 7.4, showed release to be dependent on drug loading at the earlier time points (i.e., from 1-25 hours). Release profiles were superimposable over the 200 hour study period, the effect of drug loading on release was not seen. In contrast, release of diltiazem base from PLA 109K microspheres in SGF was found to be dependent on drug loading. Release profiles were in rank order, microspheres with 10% w/w starting loading released diltiazem base at the slowest rate, microspheres with 50% w/w starting loading released diltiazem base almost immediately. Figs. 5-7 show release from 20%, 30% and 50%, respectively, diltiazem base loaded PLA 109K nanospheres at pH 1.2, pH 6.0 and pH 7.4 (prepared

according to Example 8). Because of the delivery characteristics of these microcapsules over a 24 hour period, these formulations are appropriate for once-daily administration of diltiazem.

In both media, release of diltiazem base from microsphere systems prepared with the Microfluidiser was much faster when compared to release from microsphere systems prepared with the Ultra Turrax. At 168 hours, Db_9 microspheres (10% w/w loading, prepared with the Microfluidiser) had released 100% diltiazem base in phosphate buffer pH 7.4 compared to only 16.06% from Db_2 (10% w/w loading, prepared with the Ultra Turrax).

Release profiles were fitted to Equation 2. The best estimates of K by non-linear least squares and values of correlation coefficient are given below. The fits may be considered relatively good as measured by the correlation coefficients. Release appeared to be diffusion controlled.

TABLE 6: The best estimates of K by non-linear least squares and values of correlation coefficient for diltiazem base release in phosphate buffer pH 7.4

Lot No.	Drug loading % w/w	K	correlation
Db_9	5.37	0.03572	0.98990
Db_{10}	13.61	0.04064	0.99201
Db_{11}	19.56	0.04234	0.99107
Db_{12}	42.08	0.04358	0.99816

The correlation coefficients of the release data in SGF pH 1.2 were poor when plotted over the entire course of the release study, particularly at the lower drug loading of 10% and 20% w/w. The fits were considered relatively good for diltiazem release at the higher

drug loading of 50% w/w, indicating that release was diffusion controlled. When the release data for the first five hours of diltiazem release in SGF was fitted to Equation 2, the correlation coefficients were much improved, indicating that release at the earlier time points was diffusion controlled and release was subsequently controlled by degradation of the polymer. This may be due to the fact that at the lower drug loadings there was less drug at or near the surface of the microspheres; therefore, there was less drug accessible to the dissolution medium. Polymer degradation must have occurred in order to allow drug release. Values of K were found to be proportional to drug loading. As the loading increased from 5.37% w/w (Db_9) to 42.08% w/w (Db_{12}), the value of K increased from 0.03572 to 0.04358 in phosphate buffer and from 0.07678 to 0.44293 for release in the first five hours in SGF.

Table 7: The best estimates of K by non-linear least square and values of correlation coefficient for diltiazem base release in SGF pH 1.2

Lot No.	Drug loading % w/w	Release in SGF - Hours 0-200		Release in SGF - Hours 1-5	
		K	correlation	K	correlation
Db_9	5.37	0.03620	0.82297	0.07678	0.98313
Db_{10}	13.61	0.08651	0.90619	0.12027	0.94174
Db_{11}	19.56	0.17499	0.93772	0.18784	0.95852
Db_{12}	42.08	0.44293	0.96204	0.44293	0.96204

EXAMPLE 10

Diltiazem in-vivo studies

Diltiazem nanoparticles are prepared in accordance with Examples 8 and 9 for enclosure into a capsule (e.g., gel capsule). Diltiazem HCl (USP Grade) is used as the active ingredient. Poly-D, L-lactide (R206 Boehringer Ingelheim), molecular weight 109,000,

inherent viscosity of 1.0, is used as the biodegradable polymer. Potency of the capsules is determined using high performance liquid chromatography in accordance with the method detailed in the USP.

Release characteristics of the test product are established prior to the study in accordance with the Paddle Method of U.S. Pharmacopoeia XX at 37°C and 75 rpm. The test product is shown to exhibit the following *in vitro* release profile:

Time (hr)	% release
2	10-30
4	30-60
8	60-80
20	≥80

2 x 90 mg capsules of test product are administered to volunteers. Cardizem CD 180 mg (Marion Merrell Dow) is the placebo in this study. Microfluidised microcapsules/nanoparticles, as prepared in accordance with Example 8, are used in preference, since the Z average mean diameter ranges (294-310 nm) and starting loading (10 to 50% w/w) provide characteristics which are most preferable for use in the preparations, with suitable release profiles for once-daily administration

EXAMPLE 11

Captopril *in vivo* Studies

Captopril nanoparticle capsules are prepared in a manner similar to that outlined in Examples 8 and 9. Captopril (USP grade) is used as the active ingredient. Poly-D, L-lactide (R203 Boehringer Ingelheim), molecular weight 16,000 is used as the biodegradable polymer. Hydroxypropymethycellulose and/or carbopol can be used as excipients in the formulation.

The potency of the test product is determined by HPLC by the manner outlined in USP. Release characteristics of the test product are established prior to commencement of the study, using the *in vitro* test procedure described in Example 10. The test product is shown to exhibit the following *in vitro* release:

Time (hr)	% release
1	10-40
4	20-60
8	40-80
16	>80

2 x 37.5 mg captopril containing amounts of the formulation are administered to volunteers (humans). Capoten (Trade Mark) (Squibb), is used as the placebo in this study. Microfluidised microcapsules/nanoparticles, as prepared in accordance with Example 8, as indicated above, are shown to be suitable for a once-daily preparation.

Claims: -

1. A pharmaceutical formulation for the once-daily administration of a hydrophilic drug, said formulation comprising biodegradable microcapsules containing at least one low molecular weight hydrophilic drug entrapped in a biodegradable polymer and the release of the or each hydrophilic drug from the microcapsules being substantially diffusion controlled.

2. A pharmaceutical formulation according to Claim 1, wherein the microcapsules have a D 50% between about 100 nm and 900 nm.

3. A pharmaceutical formulation according to Claim 1 or 2, wherein the hydrophilic drug loading of the microcapsules ranges from about 10% to 70% by weight.

4. A pharmaceutical formulation for the administration of a hydrophilic drug, said formulation comprising biodegradable microcapsules containing at least one low molecular weight hydrophilic drug entrapped in a biodegradable polymer, said microcapsules having a D 50% between about 100 nm and 900 nm and a drug loading which ranges from about 10% to 70% by weight.

5. A pharmaceutical formulation according to Claim 4, which can be used to administer a low molecular weight hydrophilic drug to a patient on a once-daily basis so as to achieve a therapeutic effect over a substantially 24 hour period.

6. A pharmaceutical formulation according to Claim 4 or 5, wherein the release of the or each hydrophilic drug from the microcapsules is substantially diffusion controlled.

7. A pharmaceutical formulation according to any preceding claim, wherein the microcapsules have a D 50% between about 200 nm and 400 nm.

8. A pharmaceutical formulation according to any preceding claim, wherein the hydrophilic drug loading of the microcapsules ranges from about 20% to 50% by weight.

9. A pharmaceutical formulation according to any preceding claim, wherein the hydrophilic drug is selected from a calcium antagonist, a narcotic analgesic and an ACE-inhibitor and analogues and mixtures thereof.

10. A pharmaceutical formulation according to Claim 9, wherein the hydrophilic drug is selected from diltiazem, verapamil, nifedipine, nimodipine, nicardipine, hydromorphone, codeine sulfate, oxycodone, dihydrocodeine tartrate, oxycodeinone, morphine, fentanyl, sufentanil, oxymorphone, buprenorphine and captopril.

11. A pharmaceutical formulation according to Claim 9, wherein the hydrophilic drug is a mixture of nifedipine and hydromorphone.

12. A pharmaceutical formulation according to any preceding claim, wherein the polymer matrix comprises polylactide; polyglycolide; poly(lactic acid-co-glycolic acid); poly(ε-caprolactone); poly(hydroxybutyric acid); polyortho-esters; polyacetals; polydihydropyrans; polycyanoacrylates; polypeptides; cross-linked polypeptides; and steroisomers, racemic mixtures, co-polymers and polymer mixtures thereof.

13. A pharmaceutical formulation according to Claim 12, wherein the polymer matrix comprises poly-L-lactide.

14. A pharmaceutical formulation according to any preceding claim, wherein the release profile measured in accordance with the Paddle Method of U.S. Pharmacopoeia XX at 37°C and 75 rpm for the or each hydrophilic drug is substantially as follows:

a) 10-30% release within 2 hours after administration;

b) 30-60% release within 4 hours after administration;
c) 60-80% release within 8 hours after administration; and
d) $\geq$ 80% release within 20 hours after administration.

15. A pharmaceutical formulation according to any one of Claims 1-13, wherein the release profile measured in accordance with the Paddle Method of U.S. Pharmacopoeia XX at 37°C and 75 rpm for the or each hydrophilic drug is substantially as follows:

a) 10-40% release within 1 hour after administration;
b) 20-60% release within 4 hours after administration;
c) 40-80% release within 8 hours after administration; and
d) $\geq$ 80% release within 16 hours after administration.

16. A pharmaceutical formulation according to any preceding claim, wherein the microcapsules are formulated as capsules, tablets, powders capable of effervescing upon addition of water, or suspensions.

17. A method for the manufacture of microcapsules according to any one of Claims 1-15, which comprises the steps of:

a) dissolving or dispersing a low molecular weight hydrophilic drug and a biodegradable polymer in a solvent to form a mixture;

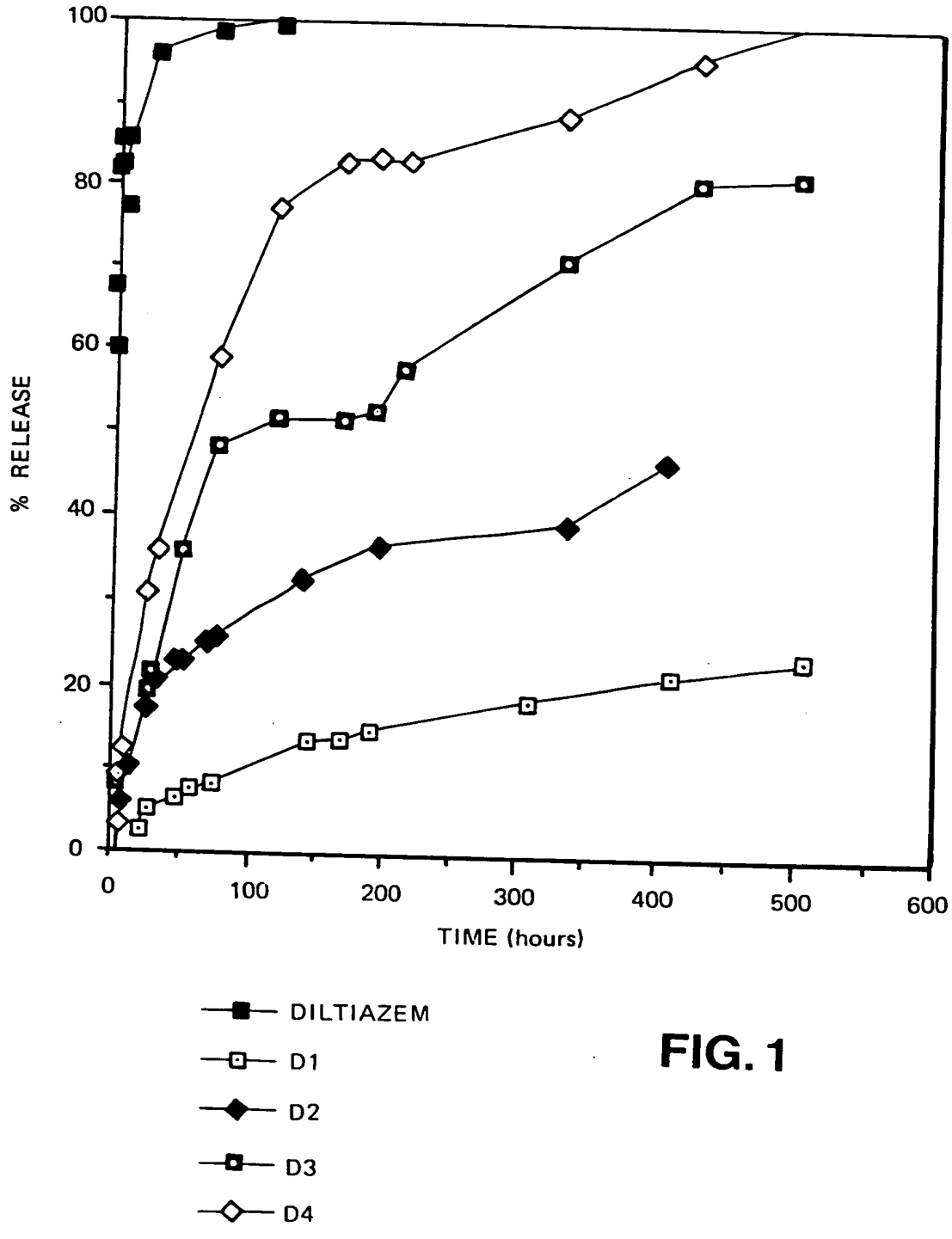
b) microfluidising said mixture into an external phase to form an emulsion in which the emulsion droplets have a mean diameter less than 1 μm; and

c) stirring said emulsion to form microcapsules having a size (D 50%) between about 100 nm and 900 nm.

Biodegradable microcapsules and method for their manufacture

Abstract

Pharmaceutical formulations for the administration of one or more low molecular weight hydrophilic drugs comprise biodegradable microcapsules containing at least one such low molecular weight hydrophilic drug entrapped in a biodegradable polymer, for example poly-L-lactide. The release of the or each hydrophilic drug from the microcapsule can be substantially diffusion controlled. Microcapsules suitably have a D 50% between about 100 nm and 900 nm and a drug loading of the order of about 10% to 70% by weight. The formulations can be used to administer low molecular weight hydrophilic drugs to a patient on a once-daily basis so as to achieve a therapeutic effect over a substantially 24 hour period. The hydrophilic drug can be a calcium antagonist, a narcotic analgesic or an ACE-inhibitor and analogues and mixtures thereof.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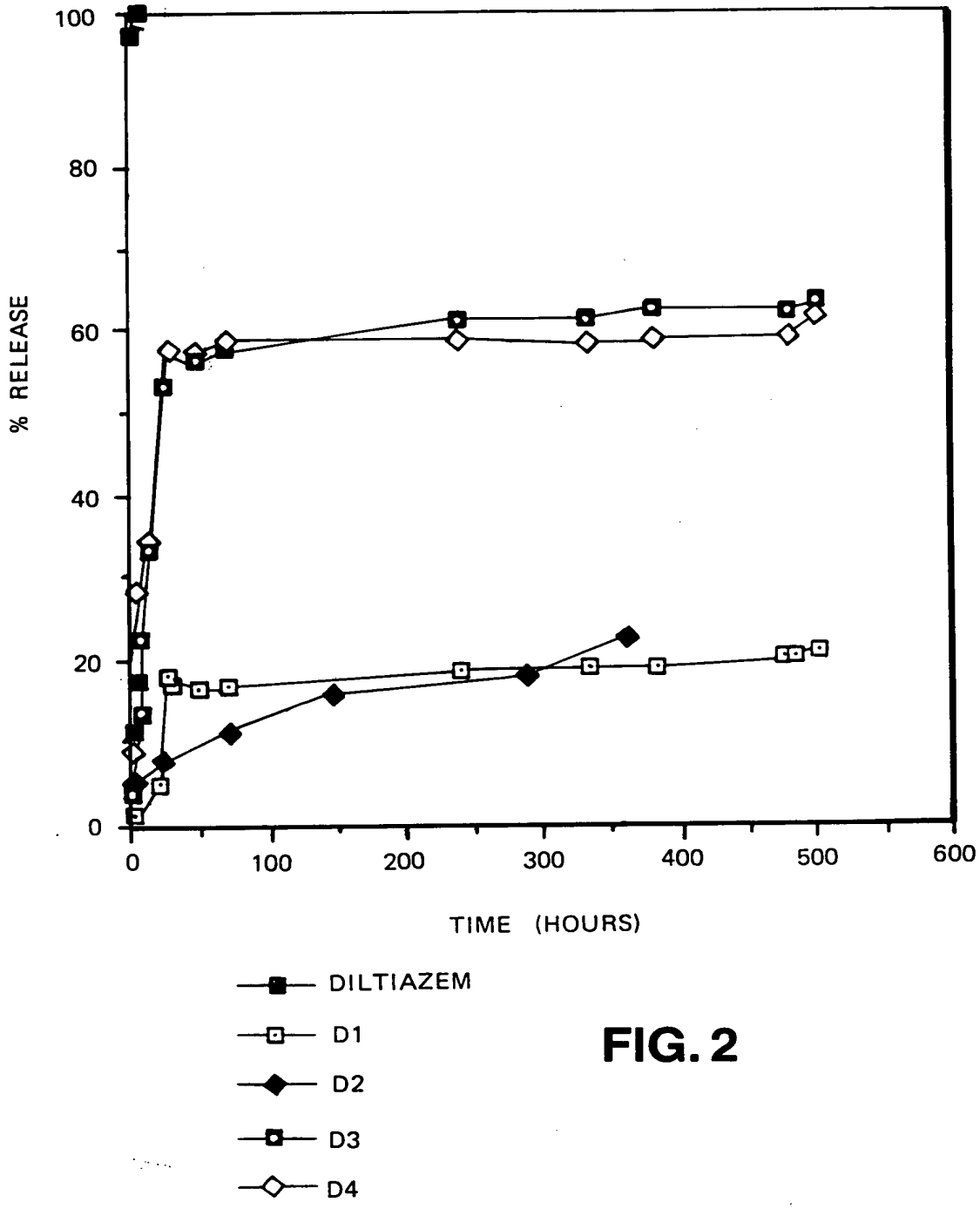
TIME (hours)
DILTIAZEM
D1
D2
D3
D4

FIG. 1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TIME (HOURS)
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D3
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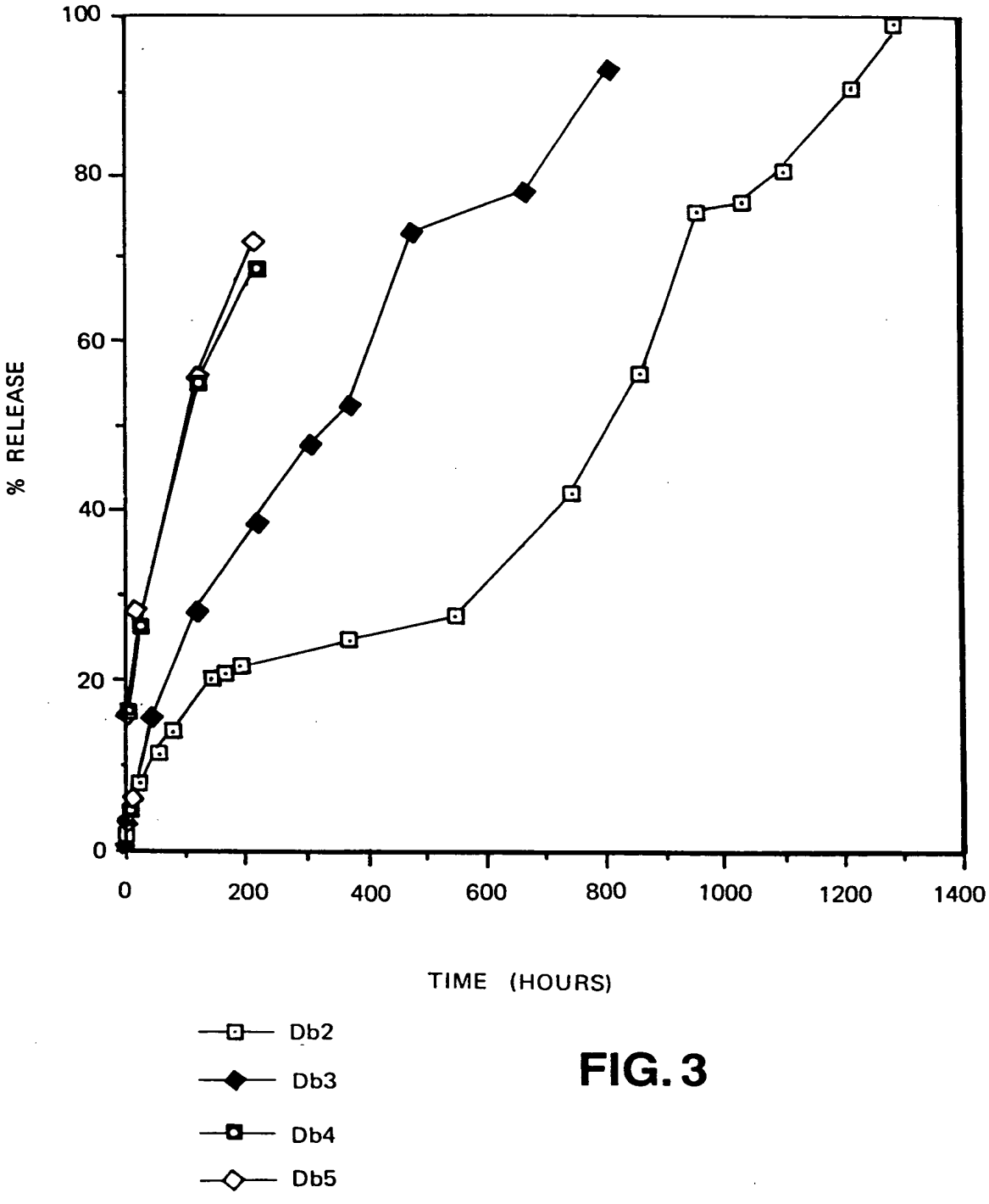
FIG. 2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TIME (HOURS)
Db2
Db3
Db4
Db5

FIG. 3

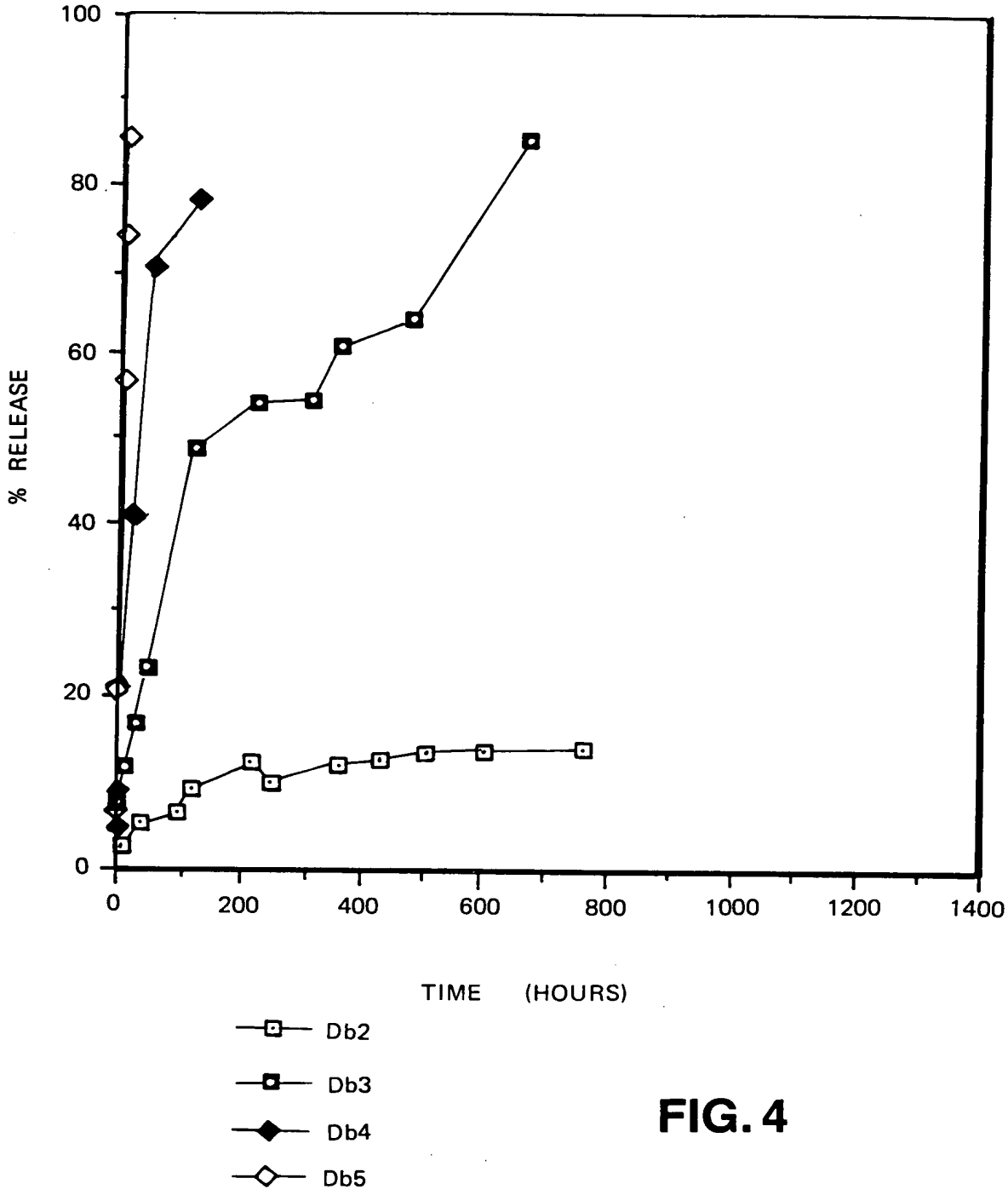
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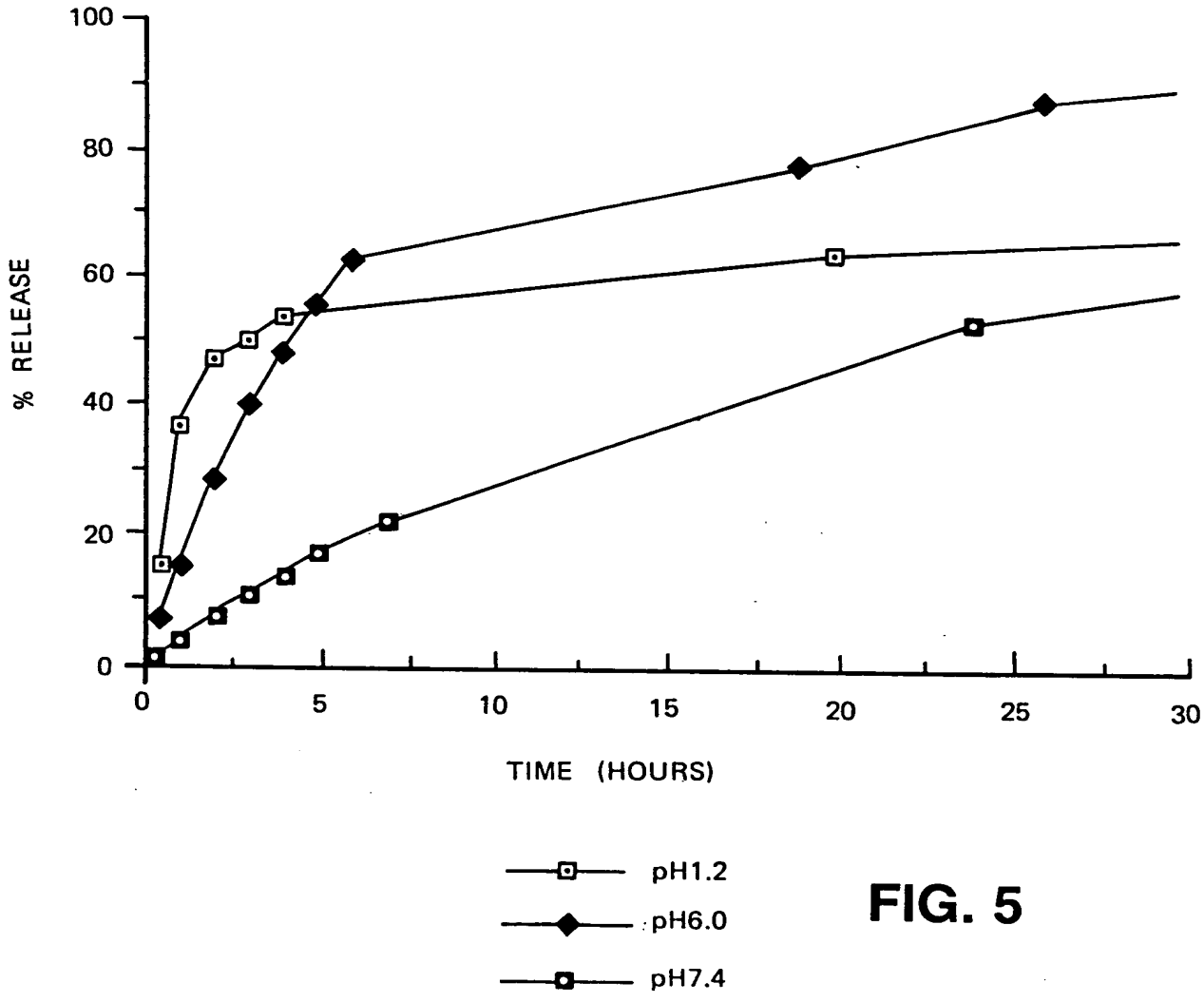
FIG. 4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15
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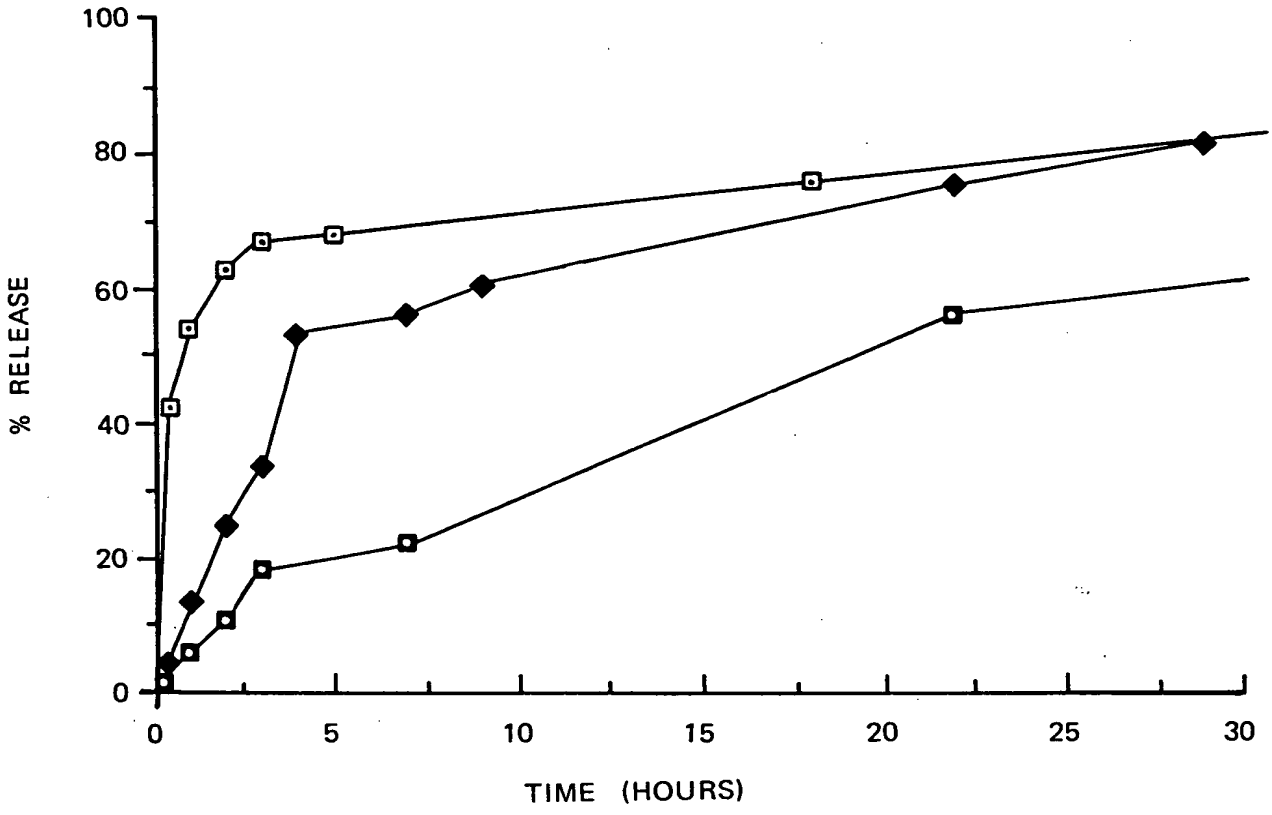
25
30
TIME (HOURS)
pH1.2
pH6.0
pH7.4

FIG. 5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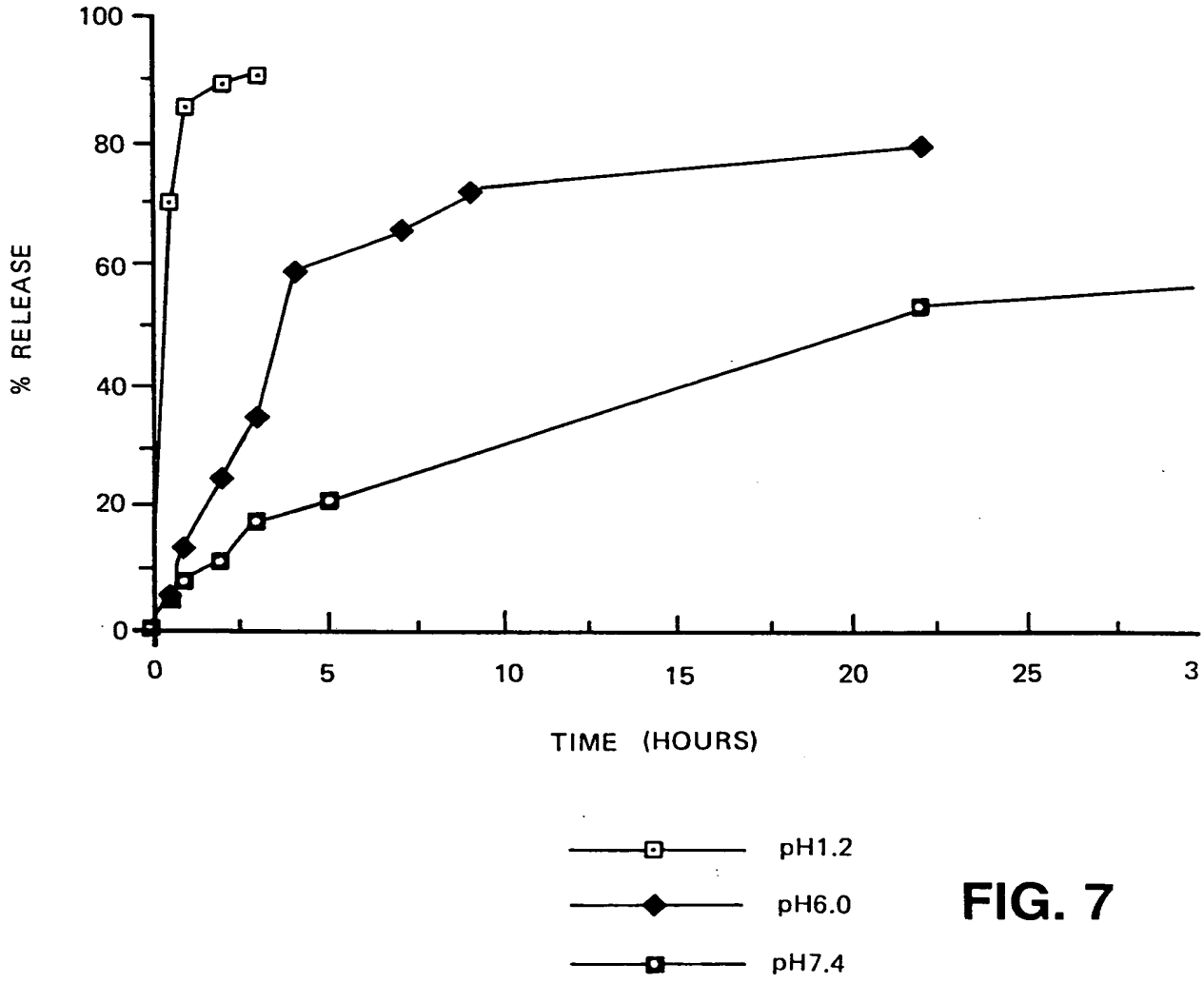
TIME (HOURS)
pH1.2
pH6.0
pH7.4

FIG. 6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80
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40
20
0
% RELEASE
0
5
10
15
20
25
3
TIME (HOURS)
pH1.2
pH6.0
pH7.4

FIG. 7